NifQ and NifO are essential to express nitrogenase activity in the presence of nitrate in *Azotobacter vinelandii.*

Jiménez-Vicente E[1], Barahona E[1], Wilcoxen J[2], Navarro-Rodríguez M[1], Buesa JM[1], Britt D[2], Rubio LM[1].

[1] Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid, Pozuelo de Alarcón, 28223 Madrid (Spain). E-mail: emilio.jimenez@upm.es, [2*] Department of Chemistry, University of California–Davis, One Shields Avenue, Davis, CA 95616 (USA).

In the presence of nitrate, *Azotobacter vinelandii* is able to assimilate nitrogen by using nitrogenase and nitrate reductase/nitrite reductase pathways simultaneously. Nitrogenase and nitrate reductase are Mo-enzymes containing FeMo-co and Mo-MGD at their active sites, respectively. In order to optimize the use of Mo, a scarce metal in nature, regulation of Mo distribution between both enzymes must be strictly controlled during nitrogen assimilation processes.

The *nifO* and *nifQ* genes are grouped together with *nifB*, *fdxN* and *rhdN* in one transcriptional unit. It has been shown that *nifO* and *nifQ* expression levels change antagonistically depending on the presence of Mo in the medium (Rodriguez-Quinones 1993). In addition, the *nifO* mutant exhibits a Nif- phenotype in the presence of nitrate, whereas *nifO* overexpression lowers nitrate reductase activity (Gutierrez, J.C. 1997). The *nifQ* mutant is unable to fix N_2 unless growth medium is supplemented with 1000-fold excess of Mo. Importantly, NifQ has been characterized as the physiological Mo donor to a NifEN/NifH complex during FeMo-co synthesis. (Hernandez, J.A. 2008).

We aimed to understand the relationship between NifO and NifQ during expression of nitrogenase activity in presence of nitrate in *A. vinelandii*. The *nifQ* mutant was unable to fix N_2 in the presence of nitrate, independently of the level of Mo in the medium. In contrast *nifQ* mutant showed enhanced nitrate reductase activity. Analysis of nitrogenase and nitrate reductase activities demonstrated that the *nifQ* overexpressing strain exhibited lower nitrogenase activity and higher nitrate reductase activity than wild-type when grown diazotrophically in the presence of nitrate, a phenotype similar to the *nifO* mutant (Gutierrez, J.C. 1997). An antagonist effect had been observed in the *nifO* overexpressing strain (Gutierrez, J.C. 1997). Simultaneous overexpression of both *nifQ* and *nifO* yielded nitrogenase and nitrate reductase activities similar to wild-type. The phenotype observed in *nifQ* overexpressing, but not in *nifOQ* overexpressing strain, points to NifO as candidate to preserve NifQ as Mo donor to nitrogenase when nitrate reductase is present.

Transcriptional expression analysis performed by RT-qPCR showed lower expression of nitrogenase structural genes in the *nifO* mutant. In contrast increased expression of nitrate and nitrite reductase structural genes was observed for both *nifO* mutant and *nifQ* overexpression strains.

Comparison between NifQ proteins isolated before and after addition of nitrate to the same culture of a *nifQ* overexpressing strain grown under diazotrophic conditions, showed NifQ cluster content alteration, resulting in decrease of $[Mo\text{-}3Fe\text{-}4S]^{3+}$ and increase of $[3Fe\text{-}4S]^{+}$ clusters. This effect of nitrate is consistent with the inability of NifQ to donate Mo for FeMo-co biosynthesis under nitrate reductase derepressing conditions.

These results revealed two Mo pathways to nitrogenase: one that can be sorted by a large excess of Mo in the medium, and a second pathway strictly dependent on NifQ and NifO that would be essential to maintain active nitrogenase while assimilating nitrate through the molybdoenzyme nitrate reductase.

References

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